Molecular origins of two-dimensional membrane viscosity measured by X-ray and neutron spectroscopies

Our body is composed of more than one trillion cells, and each cell is defined by a border that distinguishes its inner and outer regions. The boundary is a molecular membrane that is only two molecules thick and is known as the cell membrane. The variety of lipids, sugars and proteins that form cell membranes serves as a fluid matrix that facilitates interactions between individual lipids and proteins, formation of domains, and reorganization of membrane components to form multimeric signaling complexes. Understanding the transport properties in two-dimensional lipid membranes is, thus, both fundamentally interesting and biologically relevant.

Although viscosity is an intrinsic material property that characterizes the transport of momentum in a material, there have been both experimental and computational challenges in measuring two-dimensional membrane viscosity. Most of the experimental techniques use optical methods to access the membrane viscosity as a macroscopic membrane property on length scales much larger than the individual lipids, and estimates for the membrane viscosity vary by orders of magnitude depending on the experimental method used [1]. As such, there is a growing need for a more detailed understanding of the molecular origins of membrane viscosity. This is where the power of quasielastic X-ray and neutron scattering techniques was used to open new avenues to understanding the molecular origins of membrane viscosity by an extension of the length and time resolutions to cover structural dynamics at the molecular level [2].

It is not straightforward to realize a molecular understanding of the membrane viscosity without good theoretical models. In three-dimensional liquid systems, the relaxation time of the structural relaxations at a structural correlation peak follows the same temperature dependence as the liquid viscosity [3]. This relation suggests a universal feature in that the friction within liquids likely originates from the structural rearrangements of the constituent molecules. Accordingly, here we extended these studies to two dimensional systems and the measured relaxation times of the structural relaxations of the lipid acyl tails were used to calculate the membrane viscosity of a lipid bilayer [2].

We employed both Mössbauer time-domain interferometry (MTDI) [4] and neutron spin echo (NSE) spectroscopies. As a model cell membrane, we used dimyristoyl phosphatidylcholine (DMPC) bilayers at a concentration of 400 mg/mL in water. Protiated DMPC was dissolved in H_2O for the MTDI experiments while tail-deuterated DMPC dissolved D_2O was used for the NSE experiments. The experiments were performed above and below the lipid molecular melting transition temperature T_m (T_m = 24°C and 20.5°C for protiated and tail-deuterated DMPC, respectively) in order to see dynamics of lipid acyl tails both in the fluid ($T > T_m$) and gel ($T < T_m$) phases.

A priori neutron scattering experiment on TAIKAN at J-PARC showed a structural correlation peak from the lipid acyl tails at the scattering vector transfer of $q \approx 1.5$ Å^{-1}, where $q = 4\pi\sin(\theta/2)/\lambda$ with θ and λ being the scattering angle and the incident wavelength of the neutrons, respectively. The orientational order in the gel phase is higher than that in the fluid phase, and a sharper peak was observed in the gel phase [2]. Further dynamics measurements were performed at this q to measure the dynamics of the acyl tail correlations at a distance of about 5 Å. Synchrotron radiation from SPring-8 **BL09XU** was used to record MTDI signals from the samples at temperatures mainly in the gel phase. This is because the time coverage of the MTDI was from ≈10 ns up to ≈300 ns which allowed us to measure the slowest dynamics in the membrane gel phase that were otherwise inaccessible with other experimental techniques. On the other hand, NSE was used to measure relaxations both in the fluid and gel phases as the time coverage of the NSE was from

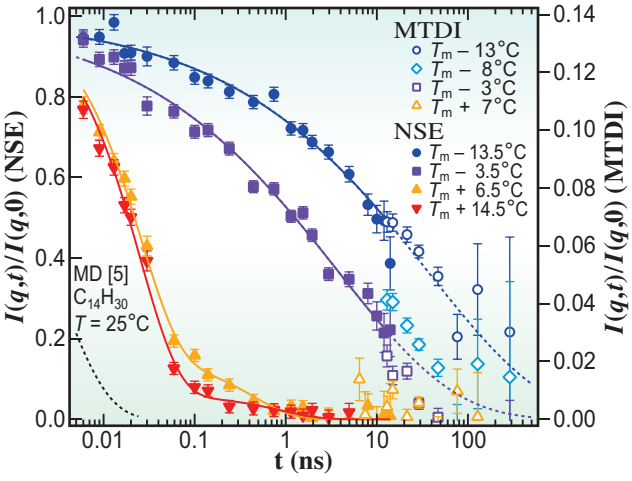

Fig. 1. Normalized intermediate scattering functions measured by the MTDI and NSE. The MTDI data are shown by open symbols on the right axis, while the NSE data are shown by full symbols on the left axis. The scale of the MTDI data is shifted to match the NSE data. The black dashed line shows molecular dynamics simulation results for tetradecane [5]. Error bars represent ±1 standard deviation in this article.

5 ps up to 16 ns on the NGA-NSE at NIST. Both these techniques measure normalized intermediate scattering function, $I(q,t)/I(q,0)$, of the lipid acyl tail correlations.

We observed fast relaxation dynamics in the fluid phase ($T > T_m$) compared to those in the gel phase ($T < T_m$) as shown in Fig. 1. In the fluid phase, two relaxation processes were identified, where the majority component had a faster relaxation time of ≈0.03 ns, and the second minor component had a slower relaxation time of ≈0.5 ns. The faster relaxation time that relates to the structural rearrangements of the individual DMPC lipid acyl tails was about an order of magnitude slower than the structural relaxation dynamics of the analogous three-dimensional linear alkane (tetradecane, $C_{14}H_{30}$), where a computer simulation result showed a relaxation time of about 0.003 ns [5]. The two-dimensional confinement and orientational ordering of the $C_{14}H_{30}$ lipid tails as well as the restricted motions due to the binding of one end of the tail to the headgroup significantly slows the chain relaxations. The slower relaxation time originates from the entire lipid escaping from the cage and rearranging with the neighboring lipid molecules, and its origins were confirmed by comparing the molecular diffusion time measured by nuclear magnetic resonance spectroscopy with the present results. This result indicates that the lipid molecules are moving out of their molecular cages on time scales of 0.5 ns.

Meanwhile, when the temperature is decreased below T_m, the relaxation dynamics are significantly slowed down to ~10 ns to ~100 ns depending on temperature. Interestingly, the data clearly show that the molecular motions persist even as the temperature is lowered into the gel phase, and the membranes are increasingly solidified at the molecular level as the temperature decreases further. However, we were not able to resolve the two relaxation modes seen in the fluid phase in the MTDI and NSE data for the lipid gel phase, indicating that the relaxation dynamics became much more heterogeneous.

Our estimates for the membrane viscosity, η_m, calculated from the structural relaxation times are summarized in Fig. 2. In the fluid phase, the slower relaxation gives a membrane viscosity on the order of 1 nPa·s·m, which falls in the middle of broadly distributed η_m values for DMPC in literature [1]. This result indicates that the membrane viscosity has a clear relationship with the lipid molecular structural relaxations. In the present study, we estimated membrane viscosity in the gel phase for the first time. The estimated membrane viscosity in the vicinity of T_m is about 10 times larger than that in the fluid phase, and significantly increases with decreasing temperature.

In summary, the combination of MTDI and NSE accessed the structural relaxation dynamics of lipid acyl tails over more than 4 orders of magnitude in time. This is a unique opportunity offered by the combination of the X-ray and neutron spectroscopic techniques and provided new insights into the collective acyl tail dynamics. The slower relaxation of the two modes in the fluid phase relates to the structural relaxation of the lipid molecules, and the time required for a lipid molecule to escape from its molecular cage is relevant to determining the macroscopic membrane viscosity. The present results provide new insights into how the individual lipid molecule motions can influence the membrane viscosity and may one day lead to future applications in drug discovery and membrane function control.

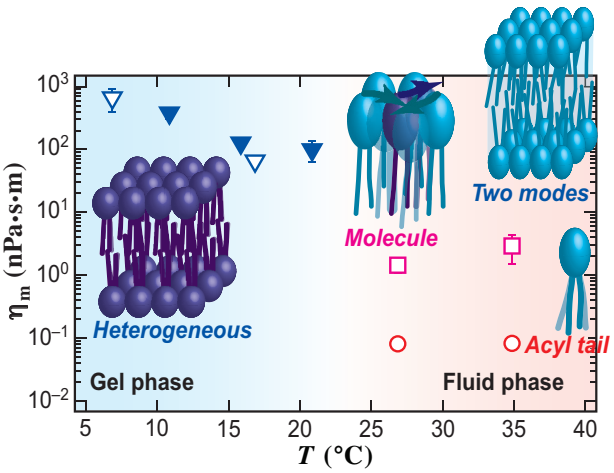

Fig. 2. Estimated membrane viscosity, η_m, from the structural relaxation time measured by the MTDI (full symbols) and NSE (open symbols) experiments. Schematic illustrations show molecular origins of the estimated η_m. Viscosity estimated from the slower relaxation in the fluid phase should correspond to apparent membrane viscosity, which appears around the middle of a large distribution of estimated η_m in literature. [1]

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